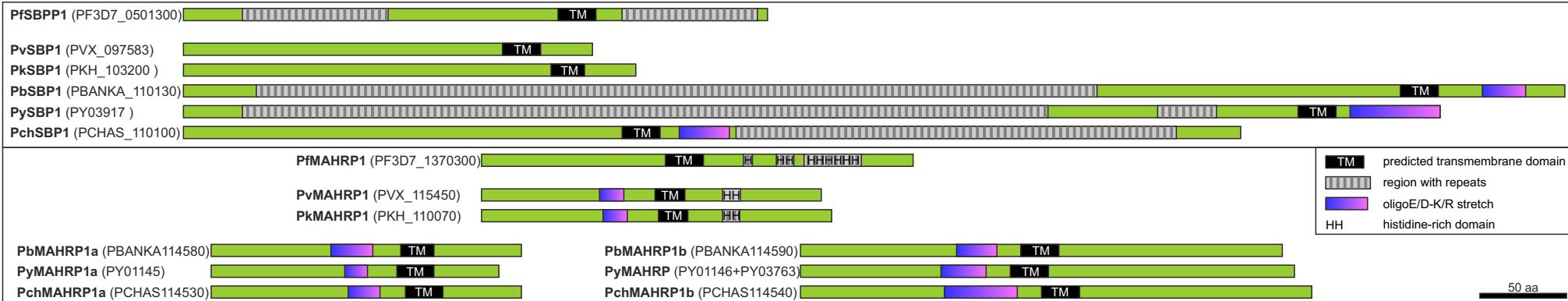


a**b**

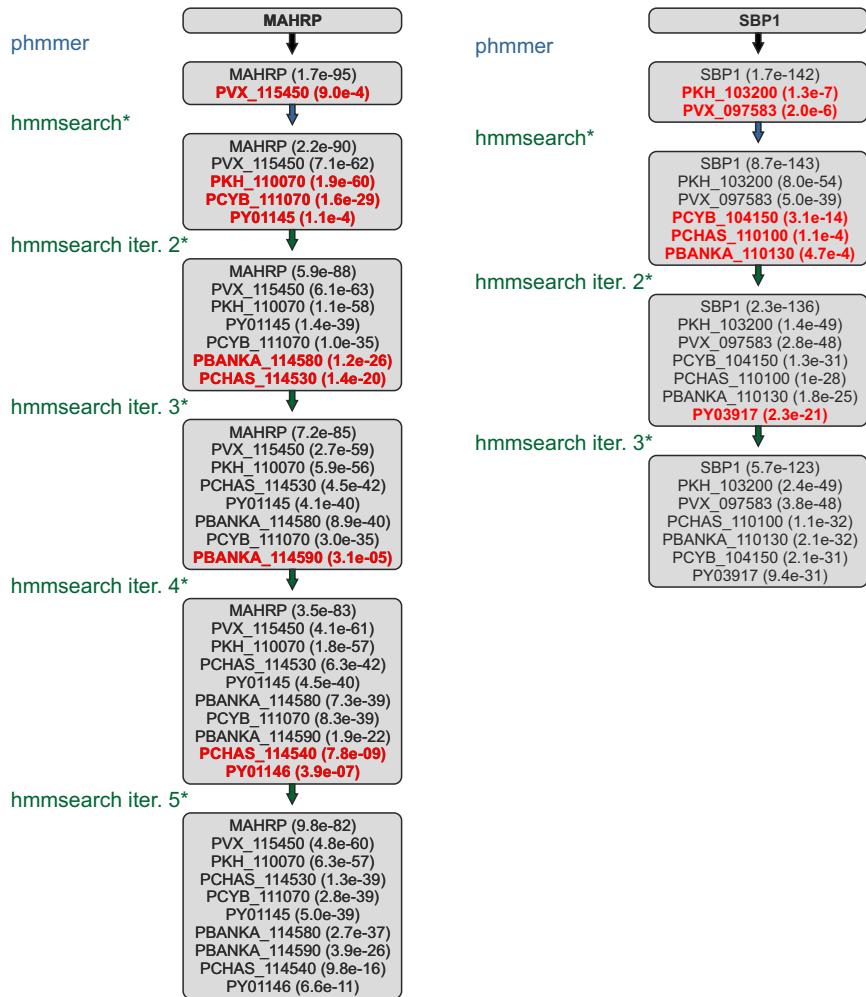
	PfaSBP1	PknSBP1	PviSBP1	PcySBP1	PchSBP1	PbeSBP1	PyoSBP1
PfaSBP1	---	51.0	50.0	39.2*	44.4	45.3	49.8
PknSBP1	26.0	---	78.0	62.5*	45.5	40.2	45.8
PviSBP1	24.5	62.9	---	76.2*	52.5	42.8	46.9
PcySBP1	19.2*	49.2*	62.3*	---	47.1	35.3	39.8
PchSBP1	18.1	22.7	28.2	26.0	---	74.1	73.8
PbeSBP1	17.9	21.7	22.5	19.1	63.2	---	75.4
PyoSBP1	19.3	23.2	24.5	21.4	62.8	64.6	---

	PfaM1	PknM1	PviM1	PcyM1	PchM1a	PbeM1a	PyoM1a	PchM1b	PbeM1b	PyoM1b
PfaMAHRP1	---	57.4	60.7	50.0	43.1	39.7	42.3	74.7	72.3	69.0
PknMAHRP1	32.4	---	79.1	69.9	45.3	40.6	47.0	45.3	40.0	37.1
PviMAHRP1	37.3	62.8	---	76.5	50.0	47.5	51.6	49.4	44.5	39.6
PcyMAHRP1	24.3	48.8	62.3	---	54.6	54.5	62.8	44.2	42.8	38.4
PchMAHRP1a	19.7	17.3	22.2	17.6	---	73.4	72.3	46.7	48.5	49.7
PbeMAHRP1a	15.6	14.7	23.0	20.9	57.2	---	84.3	41.3	46.0	43.8
PyoMAHRP1a	16.9	19.7	26.6	27.7	50.0	71.1	---	44.2	46.1	44.2
PchMAHRP1b	29.9	20.6	22.6	20.3	25.4	24.0	25.0	---	75.4	78.1
PbeMAHRP1b	30.1	20.0	18.9	18.1	23.6	24.0	26.3	60.2	---	87.6
PyoMAHRP1b	28.7	17.1	18.9	17.4	23.7	23.4	26.3	60.2	76.7	---

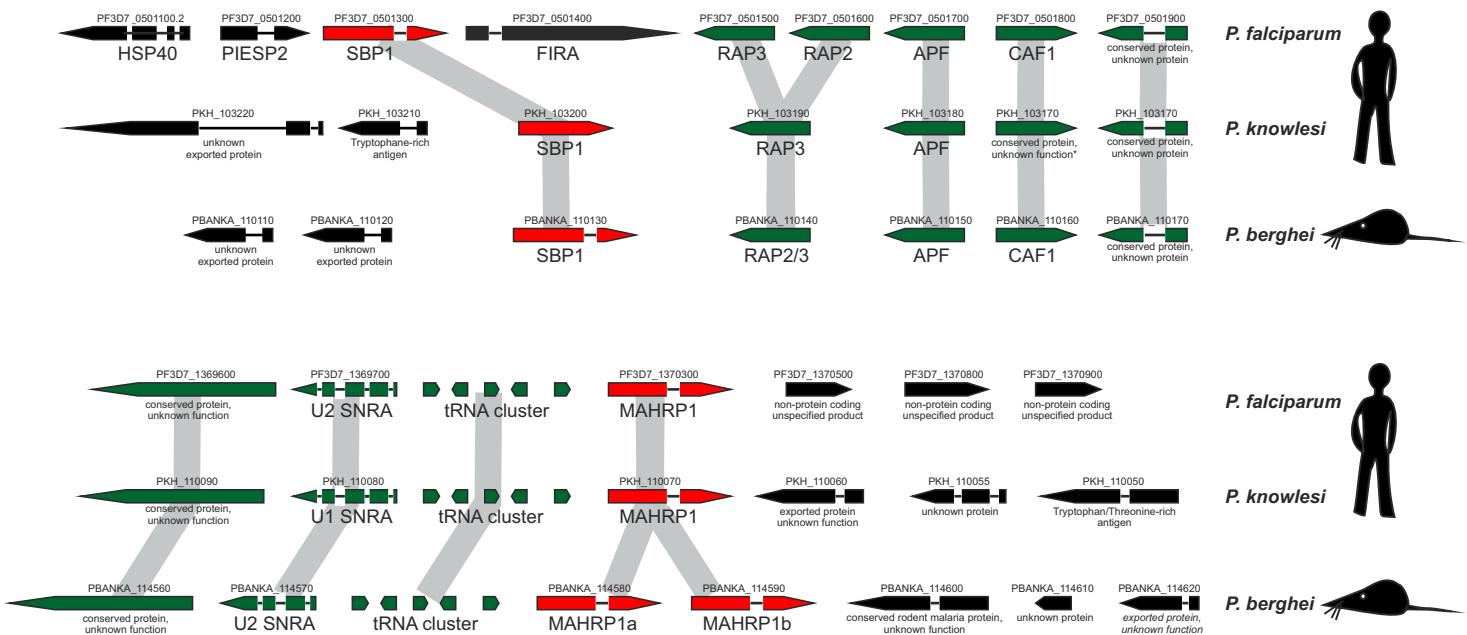
C

d

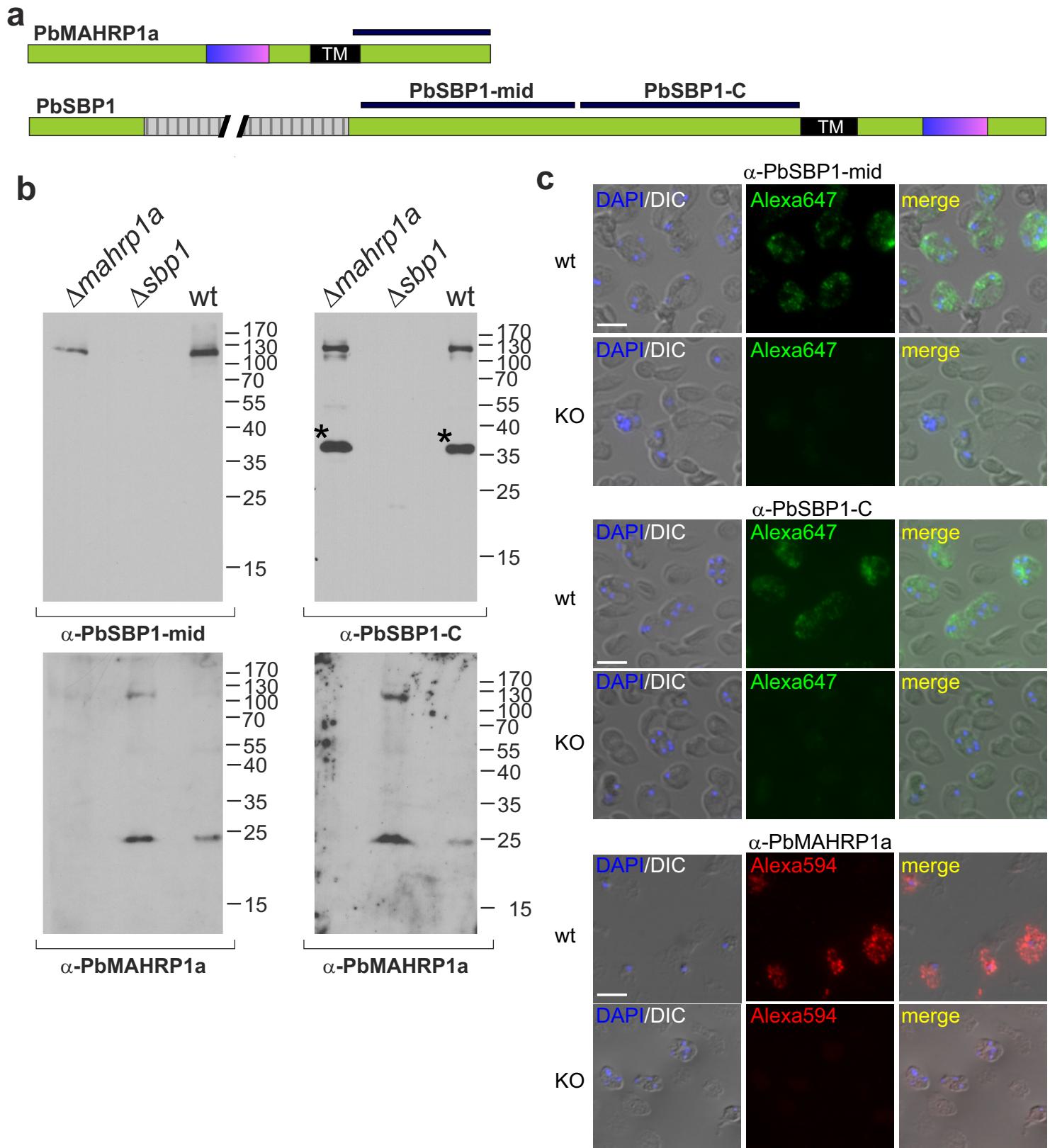
Supplementary Figure 1 | Domain structure and similarity of the identified SBP1 and MAHRP1 orthologues. (a), Graphical representation of domain structure of SBP1 and MAHRP1 orthologues. Scale 50 amino acids (aa). (b) Sequence homology tables based on the multiple amino acid alignments generated for the protein trees shown in Fig. 1c. Percentage of identical (below the diagonal) and similar amino acids (according to BLOSUM62; above the diagonal) calculated from the aligning regions are shown. Asterisk indicates that the identity/similarity scores might be biased due to incomplete amino acid sequence of PcySBP1. MAHRP1 was abbreviated to M1 in the top labels of the MAHRP table. (c,d) Alignments used to generate the phylogenetic trees in (a) for SBP1 (c) and MAHRP1 orthologues (d). Amino acids are highlighted as follows: A,V,L,I,M,F,W (red); G,P,C,Y,H,Q,N,S,T (green); K,R (magenta); E,D (blue).



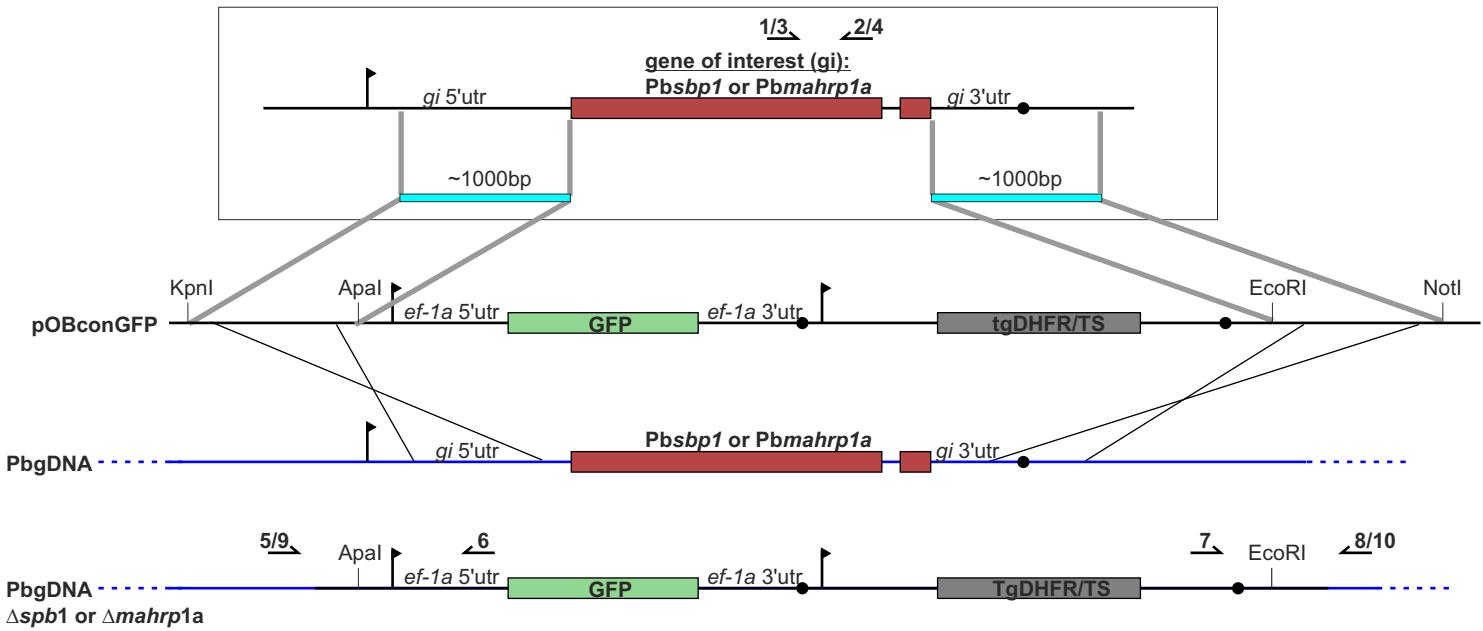
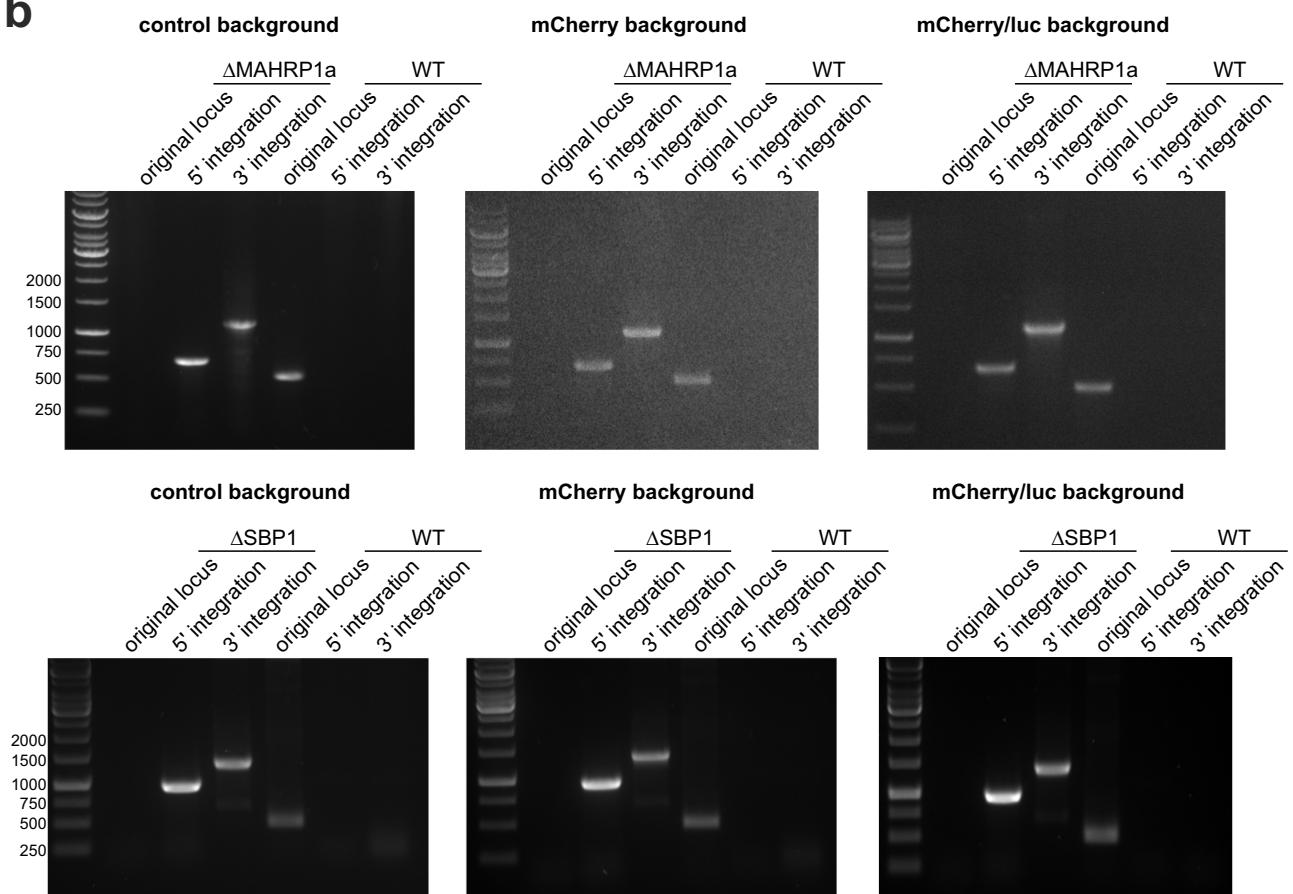
Supplementary Figure 2 | A jackhmmer search confirms the orthology of the SBP1 and MAHRP1 proteins of different *Plasmodium* species identified by BLAST searches. The search strategy is indicated on the left of the boxes; PlasmoDB gene IDs of newly identified molecules per step/iteration (iter.) are shown in red (corresponding e-values are given in brackets). An e-value inclusion threshold of 0.001 was used (indicated by an asterisk).



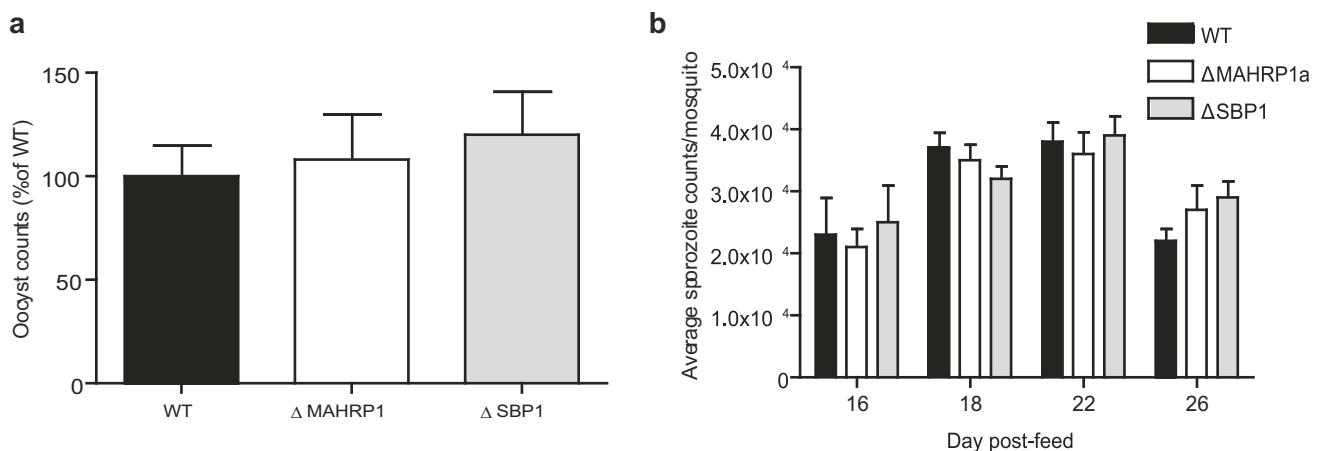
Supplementary Figure 3 | The *sbp1* and *mahrp1* genes of different *Plasmodium* species have a syntetic location in the genomes. Genomic regions harbouring the *sbp1* and *mahrp1* genes are shown for selected *Plasmodium* species. Syntenic genes with clear orthology (as defined in PlasmoDB) are shown in green, *sbp1* and *mahrp1* genes are shown in red. Synteny is indicated by gray lines. Non-syntenic genes (and genes without clear orthologues in other species) are shown in black. The size of open reading frames, introns and the intergenic regions are not shown to scale.



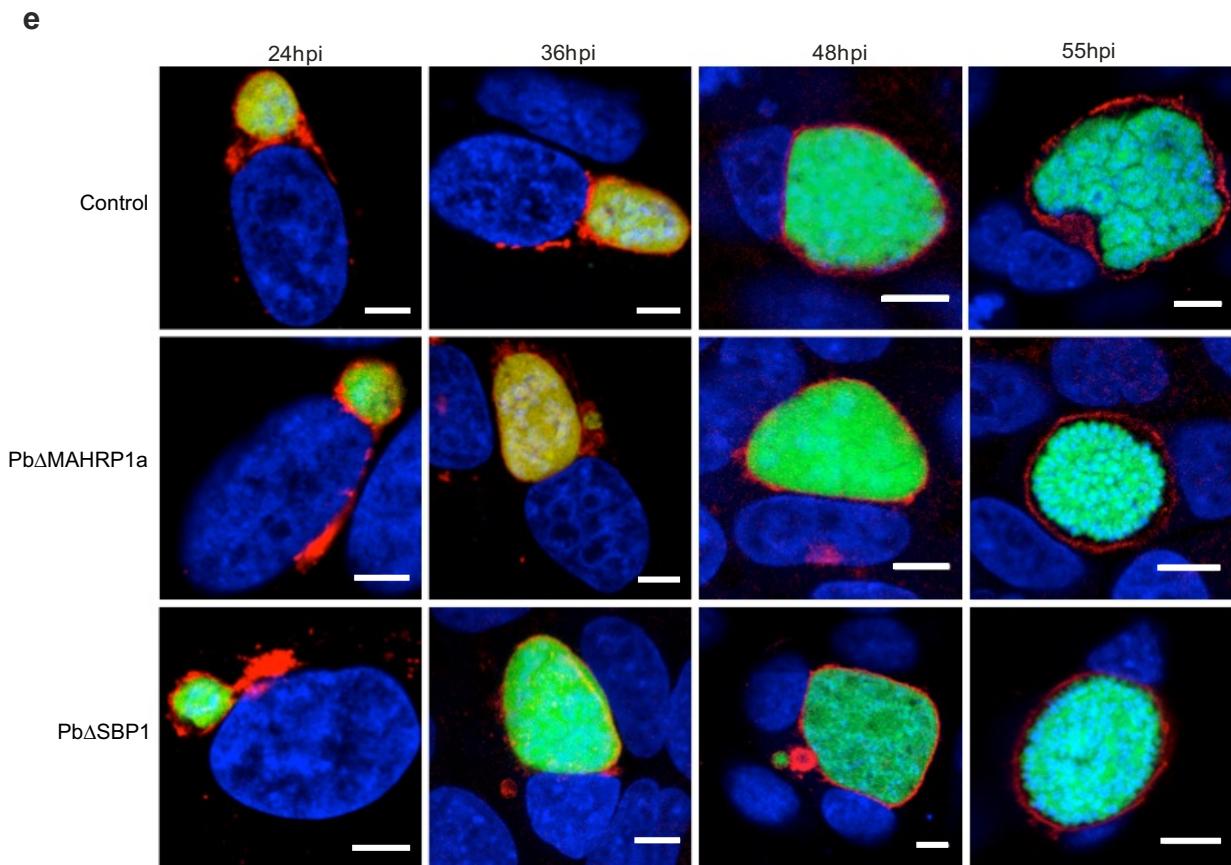
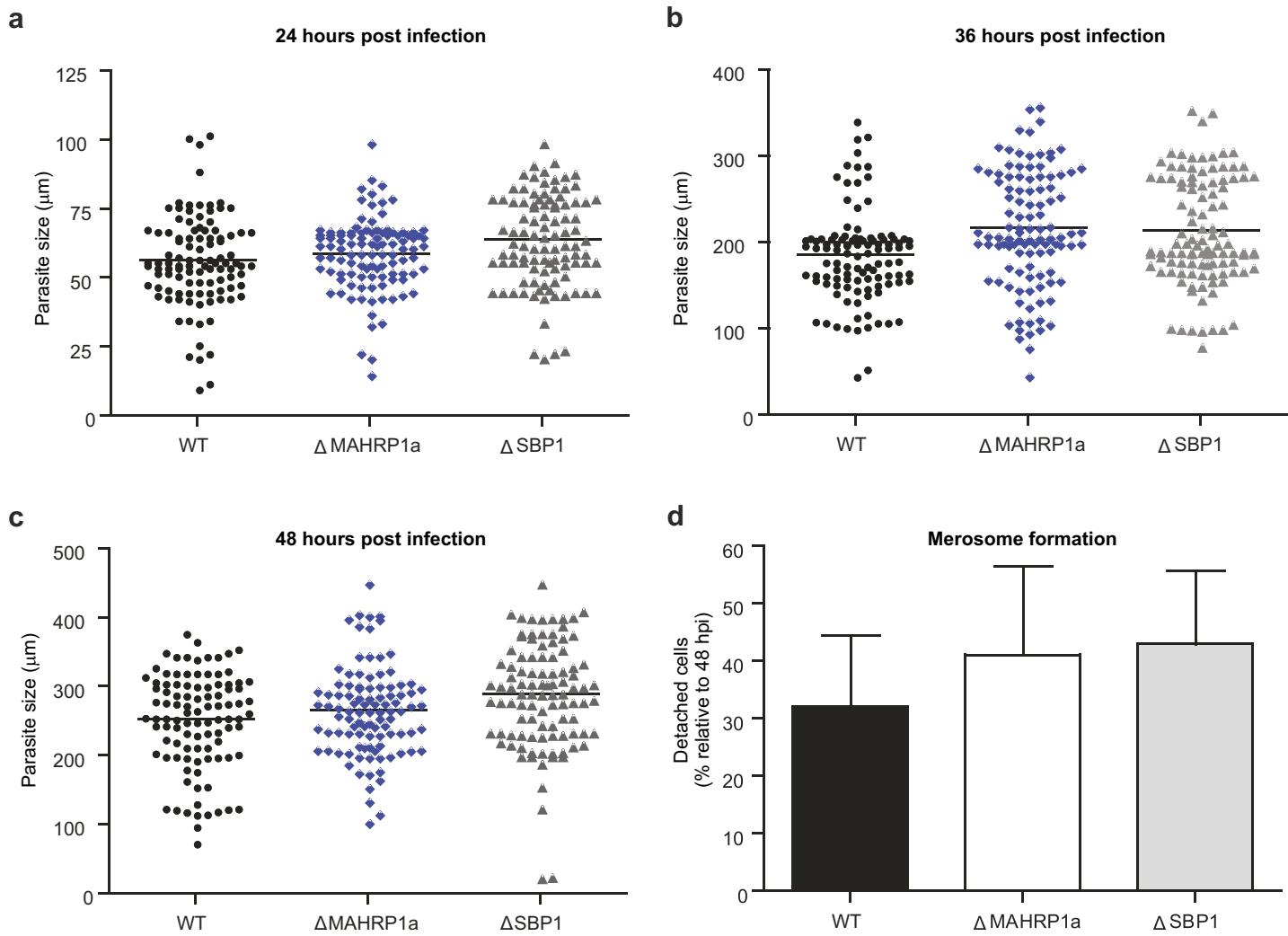
Supplementary Figure 4 | Specificity of antisera raised against PbSBP1 and PbMAHRP1a. (a) Schematics of PbMAHRP1a and PbSBP1 (repeat region truncated) with the regions used to raise antisera indicated as black bars. Two antisera were raised against two non-overlapping regions of PbSBP1 (PbSBP1-mid and PbSBP1-C). (b), Western blot analysis of *P. berghei* protein extracts from purified blood stages of wild type (wt), and Pb Δ sbp1 and Pb Δ mahrp1a gene-deletion parasites using the different antisera. Molecular weight standards are in kDa. The asterisk denotes a fragment detected only with α -PbSBP1-C, indicating C-terminal processing of PbSBP1. This is a PbSBP1-specific fragment, as it is not present in Pb Δ sbp1 gene-deletion parasites. The signal with α -MAHRP1a and α -PbSBP1 is used as loading control for Pb Δ sbp1 and Pb Δ mahrp1a gene-deletion parasites, respectively. (c), IFA analysis of blood stages of wt and Pb Δ sbp1 and Pb Δ mahrp1a gene-deletion parasites (KO) using the different antisera. Specific staining is only detected in wt parasites, confirming the specificity of the antisera. Merge: merge of all three channels. Size bars: 5 μ m.

a**b**

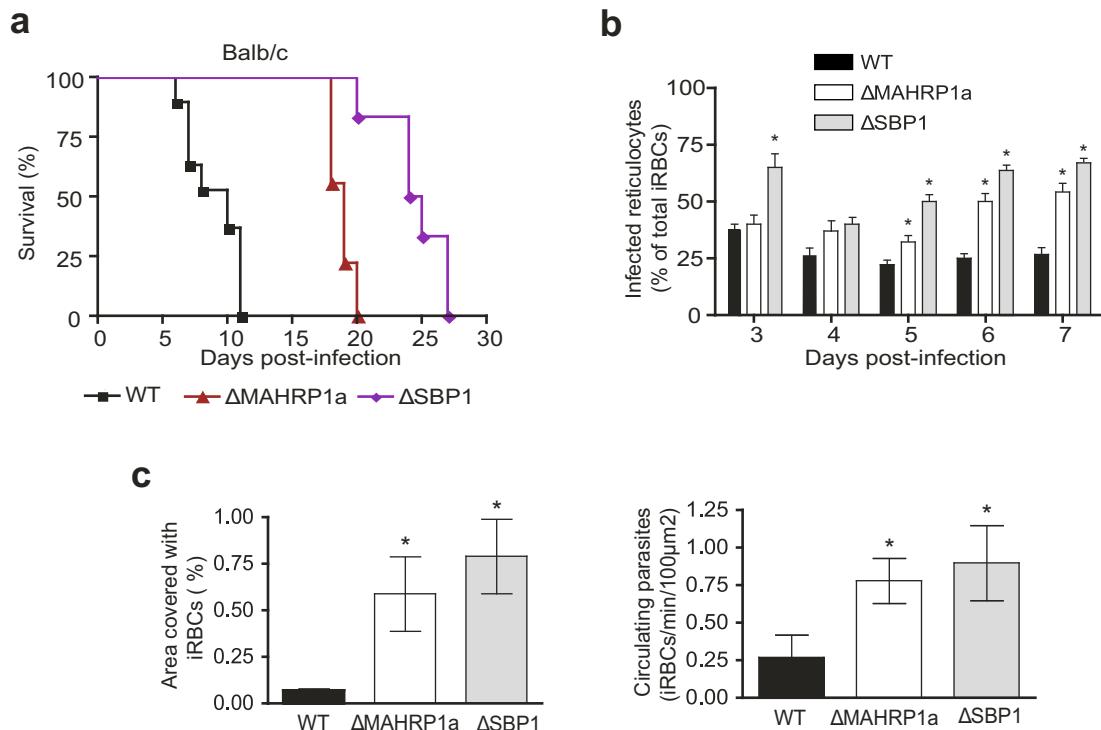
Supplementary Figure 5 | Generation of the Pb Δ sbp1 and Pb Δ mahrp1a parasites. (a) The box shows a schematic of the target genes with the targeting regions inserted into the pOBconGFP gene deletion construct shown below. PbgDNA shows the unmodified genomic target region and below the genomic locus after integration of the gene deletion construct pOBconGFP. Numbered arrows: primers used in (b). (b) Diagnostic PCR-analysis confirm correct integration of the pOBconGFP construct in the different cloned lines of Pb Δ sbp1 and Pb Δ mahrp1a gene-deletion parasites. Primers 1-4 were used to detect the original locus, primers 5/6 and 9/6 for 5' integration and primers 8/7 and 10/7 for 3' integration. Primers are indicated by arrows.



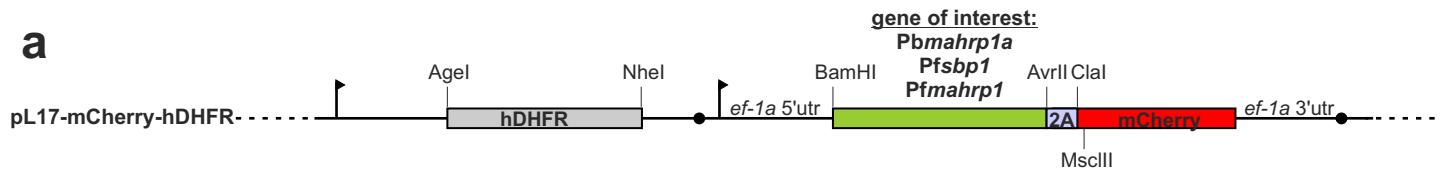
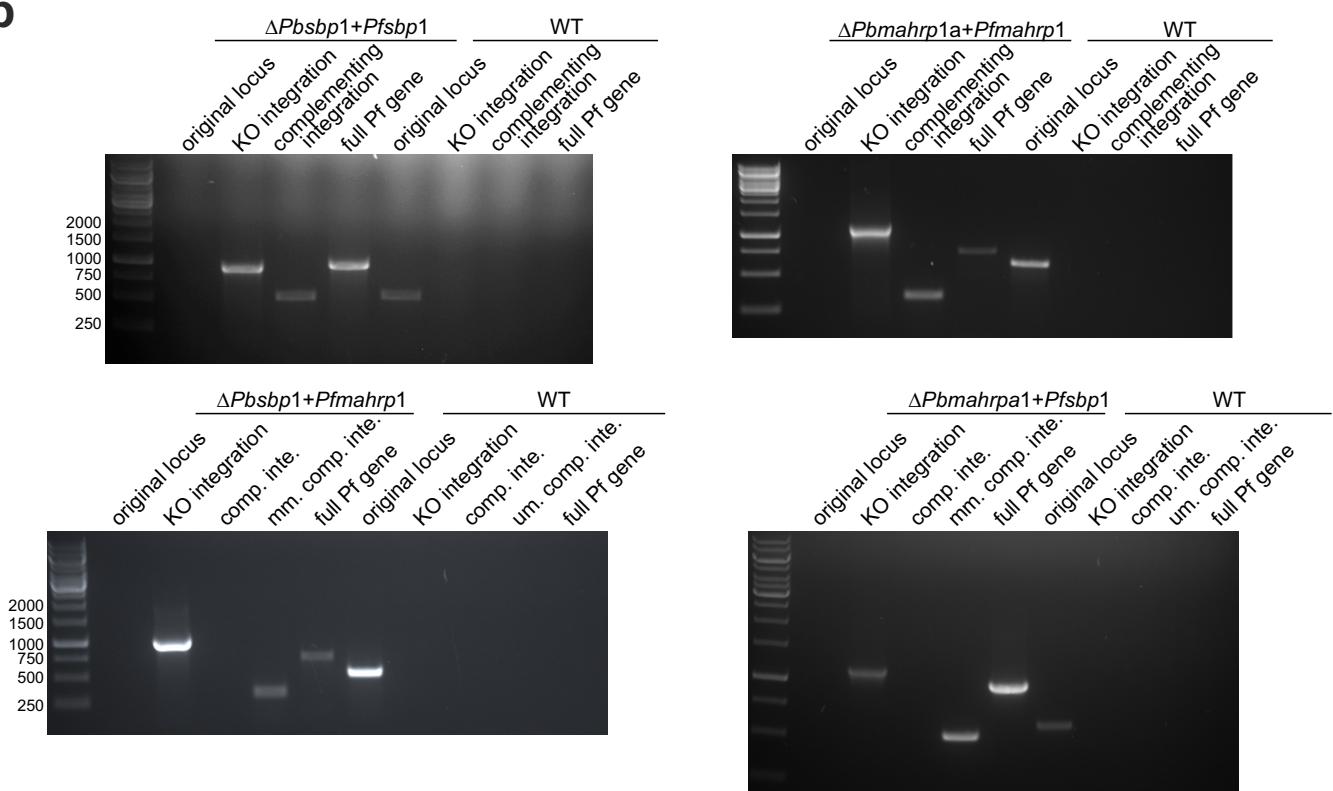
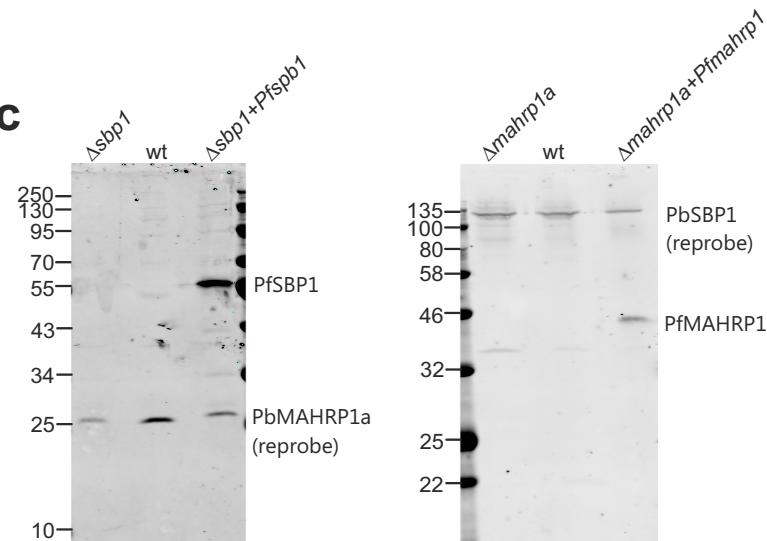
Supplementary Figure 6 | *PbΔsbp1* and *PbΔmahrp1a* gene-deletion parasites show development in the mosquito similar to wt parasites. (a) No significant difference exists in oocyst production between mosquitoes infected with wild type (wt) and gene-deletion mutant parasites ($P = 0.1$, Student's *t*-test). Oocyst numbers (n=60 mosquitoes) are shown relative to wt (%). (b) No significant difference in sporozoite production (numbers and timing; $P = 0.08$, Student's *t*-test) between mosquitoes infected with wt and gene-deletion mutant parasites. Salivary gland sporozoites were counted at days 16-26 post blood meal (n=60 mosquitoes).



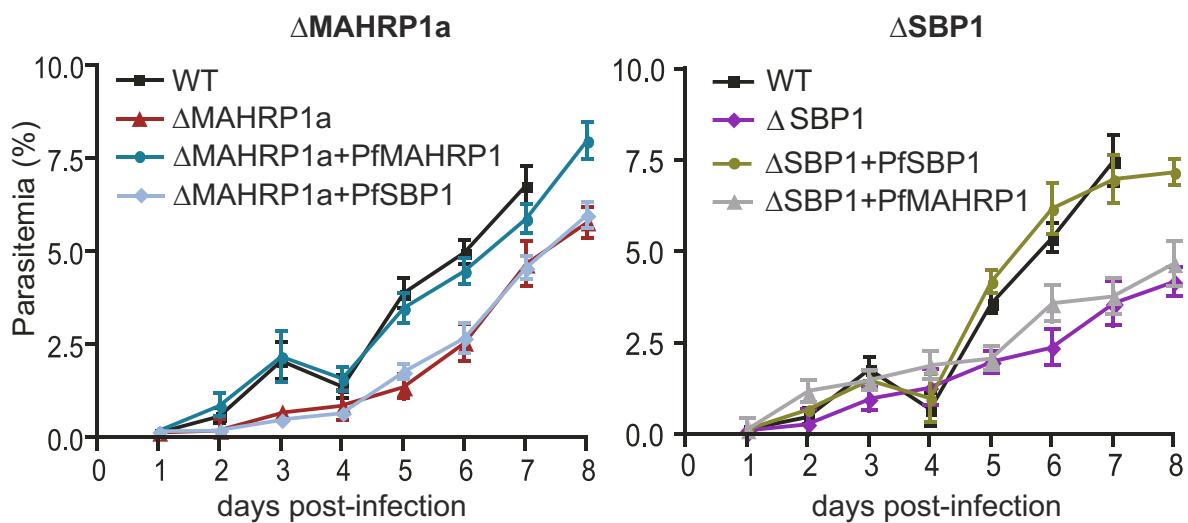
Supplementary Figure 7 | Pb Δ sbp1 and Pb Δ mahrp1a parasites show development in hepatocytes similar to wt parasites. (a-d) Individual sizes of liver-stage parasites in cultured HeLa cells at different time points post infection (p.i.) with wt and mutant sporozoites. Sizes were measured of 100 parasites per well (triplicate wells). (d) Merosome formation (detached cells), representing successful completion of liver stage development at 65 h p.i.. Detached cell numbers are expressed as % of parasites present at 48h p.i in the same well (triplicate wells). (e) Similar development of wt and mutant liver stages as visualised by confocal microscopy at various hours p.i.. Parasites were stained with the nuclear dye DAPI (blue), antibodies against the parasitophorous vacuole membrane were anti-UIS4 (red; 12-24 h); anti-exp1 (red; 36-56 h) and anti-GFP (cytoplasm; green) (GFP).



Supplementary Figure 8 | Features of blood stage infections in mice infected with wt, *PbΔsbp1* and *PbΔmahrp1a* parasites. (a) Significantly prolonged survival of Balb/c mice infected with mutant parasites compared to wt ($n = 15$ mice per group, logrank test $p < 0.001$; Kaplan-Meier curves). (b) Reticulocyte versus normocyte preference of wt, *PbΔsbp1* and *PbΔmahrp1a* parasites in C57B/6 mice at days 3-7 after infection. Tropism was determined in Giemsa stained blood films. A significantly higher number of gene-deletion mutant parasites were identified within reticulocytes, as opposed to wt parasites ($P < 0.001$, Student's *t*-test). c, Quantification of the presence of schizont-infected RBC in spleens of UBC-GFP mice at 22 hours after establishing synchronised infections of wt and mutant parasites. Fifty fields of view of splenic tissue were observed for blood flow and parasite circulation. 50 positions were selected in every spleen, and intravital movies generated for a period of 5 minutes (2 images/s). Total area covered with iRBCs adhering to the splenic tissue or circulating through splenic vasculature, was quantified. The rate of passage of circulating iRBC was measured within open circulation or vessels, and values expressed as iRBCs/ minute/ area. Experiments were performed in $n = 3$ mice per parasite line ($P = 0.001$, Student's *t*-test).

a**b****c**

Supplementary Figure 9 | Generation of the complementation of mutants. (a) Plasmid for the generation of complementation mutants. The part of plasmid of the pL17mCherry-hDHFR relevant for complementation is shown. Note that due to the skip peptide (2A), the complementing protein is without a tag that could affect its function while at the same time the complemented parasites express cytoplasmic mCherry. mCherry fluorescence therefore demonstrates complementation and is visible in addition to the cytoplasmic GFP fluorescence that is indicative of the respective gene deletion. mCherry and GFP fluorescence was microscopically confirmed for all clones used. (b) Diagnostic PCR-analysis confirms correct integration of the matched and mismatched complementation constructs in the cloned Pb Δ sbp1 and Pb Δ mahrp1a gene-deletion parasites. 'Comp. inte.', complementing integration; 'mm. comp. inte.', mismatched complementing integration; 'full Pf gene', detection of full complementing gene. To confirm integration, primers spanning the junction between the insertion and the 5' genomic region were used (primer AB_efla 5' UTR - Fw with either PfMAHRP1_int_rev or PfSBP1_int_rev, see Table S1). To confirm the presence of the full gene, primers binding at the 5' and 3' end of each gene were used (primers PfMAHRP1_full-fw with PfMAHRP1_full_rev and PfSBP1_full-fw with PfSBP1_full_rev, see Table S1). (c) Western blot showing expression of the complementing *P. falciparum* proteins in the gene deletion parasites but not uncomplemented or wt parasites. As a loading control PbMAHRP1a was detected in the Pb Δ sbp1 parasites and PbSBP1 in the Pb Δ mahrp1a parasites (by re-probing onto the same membrane).



Supplementary Figure 10 | The course of parasitemia in C57BL/6 mice infected with wt, mutant and complemented mutants. Complementation with *Pfmahrp1* and *Pfsbp1* restore a growth rate similar to that of wt *P. berghei* (n=12 mice per complemented mutant). The parasitemia of matched complemented parasites was similar to the parasitemia of wt parasites. The parasitemia of mismatched complemented parasites was similar to that of the gene-deletion mutants ($P=0.07$, Student's *t*-test).

Supplementary Table S1: Primers used in this study

Primer target	Primer sequence 5' - 3'	Purpose
PbSBP1-5utr-Kpnfw2	CAGCGGTACCGTTATGTATCTCTAAAAGGATAGAAGC	PbSBP1 KO construct
PbSBP1-5'utr-Aparv2	GCTGGGGCCCGCAATTGATTCAAGGAGCATAAGTTGATATTGATTAC	PbSBP1 KO construct
PbSBP1-3'utr-Ecorlfw	CAGCGAAATCCGGATATAAAATTATAAAAGTTATTAATGTAAG	PbSBP1 KO construct
PbSBP1-3'utr-Notrv	GCTGGCGGCCGCCGAATTAAATCTCTTAAGAGAAAGTTTTGATGC	PbSBP1 KO construct
hDHFR-F-Agel	ATACCGGTAAATGGTTGGTTCGCTAAACTGC	AB plasmid cloning
hDHFR-R-Nhel	ATGCTAGCATTAATCATTCTCTCATATACTTCAAATTGTA	AB plasmid cloning
PbMAHRP1a_fw_BamHI	CAGCGGATCCTAAAATGGCTTACACTGAAAAAGAAGGTAAAGAAG	PbMAHRP1a AB construct
PbMAHRP1a_rev_AvrII_part 2A_1	GGTCCTGGATTTCTTACATCTCACATGTTAATAAACTCCCTTCTCCCCTAGGTCAAGTTAGTTCTATGGTAGTAGTGCTCTAGTAGTAC	PbMAHRP1a AB construct
PbMAHRP1a_rev_ClaI_part-2A_part mCherry_MscI_2	GCTCTGGCATGTTATCCTCCTGCCCTGCTCACCATATCGATTGGCTGGATTTCCTACATCTCC	PbMAHRP1a AB construct
PfMAHRP1_fw_BamHI	CAGCGGATCCTAAAATGGCAGAGCAAGCAGCAGTACAACCAGAAAG	PfMAHRP1 AB cloning
PfMAHRP1_rev_AvrII	GCTCCCTAGGATTATCTTTTTCTTGTCTAATTTC	PfMAHRP1 AB cloning
PfSBP1_fw_BamHI	CAGCGGATCCTAAAATGTGTAGCGCAGCTCGAGCATTG	PfSBP1 AB cloning
PfSBP1_rev_AvrII	GCTCCCTAGGGTTCTAGCAACTGTTTGTGGATTGG	PfSBP1 AB cloning
PbMAHRP1a_C_fw_BamHI	CAGCGGATCCACATATTCAAGGATTGCACAGCATAGAACATC	PbMAHRP1a pGEX cloning
PbMAHRP1a_C_rev_Xhol	GTCGCTCGAGTCAGTTAGTTCTATGGTAGTAGTGCTCT	PbMAHRP1a pGEX cloning
PbSBP1_mid_fw_BamHI	CAGCGGATCCAAGATACAGAAAATAATTCAAGATACAGAAAAGAAATTAGATAACAGAAAAGAAATTCAAGATAAGAAAAGAAATATCTC	PbSBP1 pGEX cloning
PbSBP1_mid_rev_Xhol	GTCGCTCGAGATAAGGAGAATCAATTGCAGTTTACATTAGAACCTGGACTATCATCTCCAAGAATTGGTATTGTATTGGTTCAAGATAATTGG	PbSBP1 pGEX cloning
PbSBP1_C_fw_BamHI	CAGCGGATCCAATGCTCAACGCCAACACTGATTCAAAGTG	PbSBP1 pGEX cloning
PbSBP1_C_rev_Xhol	GTCGCTCGAGTATTAAACCTTTCCGTGAAATTCTTCC	PbSBP1 pGEX cloning
PbSBP1-bam-fw	CAGCGGATCCAAAATGGATAATCAAATATCAACTTATGCTCC	PbSBP1 pL17 cloning
PbSBP1-bam-rv	CAGCGGATCCTTTTTAAATTCTTCCGGTCTTATTGCC	PbSBP1 pL17 cloning
KpnI-PbMAHRP1a1-20-mTRAP_F	CAGCGGTACCATGGTTACACTGAAAAAGAAGGTAAAGAAGAAAGTAAATTATGCATCCCCAAGATGAATCTGCATTATATGAACATATGAATAC	mTRAP fusion cloning
KpnI-PbSBP1-1-20-mTRAP_F	CAGCGGTACCATGGATAATCAAATATCAACTTATGCTCTGAATTGCTCAAATATATTAGATTCTGCATTATATGAACATATGAATAC CGCGCCTAGGTTCGAGTGCCGCCAGAATTCTTCTTC	mTRAP fusion cloning
PbMAHRP1a- Fw (1)*	CGAGAGTGAAGAAGATAG	Diagnostic PCR WT MAHRP1a
PbMAHRP1a- Rev (2)*	ATTTTTAGGTGATGTAAGTCATGAACG	Diagnostic PCR WT MAHRP1a
PbSBP1- Fw (3)*	GAAGCAGCACAGCGGAACAGT	Diagnostic PCR WT SBP1
PbSBP1- Rev (4)*	GCAAGTAAACATAACAAAAAGGCC	Diagnostic PCR WT SBP1

PbΔMAHRP1a 5' Fw (5)*	GTCCTGAAACTAATATAAAGGCC	Diagnostic PCR MAHRP1aKO
ef1a 5' UTR - Rev (6)*	TTATTTGCACTACTGGAAAATACC	Diagnostic PCR KO
Tgdhfr 3'- Fw (7)*	TAGCGGAAATACAGAACGCTAGC	Diagnostic PCR KO
PbΔMAHRP1a 3' Rev (8)*	GCATAATTTCTGAACCATCTGG	Diagnostic PCR MAHRP1a KO
PbΔSBP1 5' Fw (9)*	AAAAATACAATCTCAAGGAGGTGC	Diagnostic PCR SBP1 KO
PbΔSBP1 3' Rev (10)*	TGCGCCGACCATCTTAGTATC	Diagnostic PCR SBP1KO
PfMAHRP1_full_fw	ATGGCAGAGCAAGCAGCAGTACAACCAGAAAG	Diagnostic PCR for full PfMAHRP1
PfMAHRP1_full_rev	ATTATCTTTTTCTTGTCTAATTTGC	Diagnostic PCR for full PfMAHRP1
PfSBP1_full_fw	ATGTGTAGCGCAGCTCGAGCATTG	Diagnostic PCR for full PfSBP1
PfSBP1_full_rev	GGTTCTCTAGCAACTGTTTGTTGGATTGG	Diagnostic PCR for full PfSBP1
AB_ef1a 5' UTR - Fw	GTTGTGAAACAAAAACG	Diagnostic PCR for AB
PfMAHRP1_int_rev	CATCAACTCTTAAAG	Diagnostic PCR for PfMAHRP1AB
PfSBP1_int_rev	GTGCCTCTGCTGCATACCAACTAAATTCC	Diagnostic PCR for PfSBP1AB

*number refers to the primers indicated in Extended Data Figure 5